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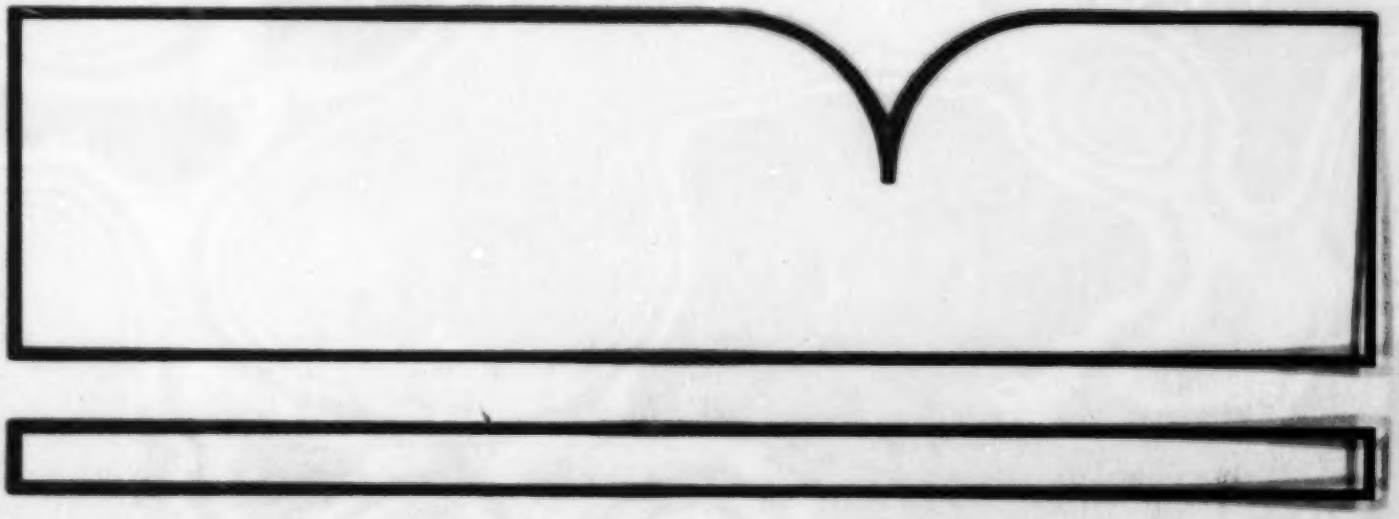

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COMPARISON OF HIGH MOLECULAR WEIGHT ORGANICS ISOLATED FROM DRINKING WATER IN FIVE CITIES

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16. ABSTRACT

Since the previously unidentifiable non-volatile fraction of the chlorinated organics in drinking water is of significant health concern, the physical and chemical characteristics of the high molecular weight organics isolated from drinking water in five cities (namely: New Orleans, Philadelphia, Miami, Seattle, and Ottumwa) were compared. From the results of this study, they may be considered as part of a single group of natural organic compounds. Although the respective molecular structures of this group of compounds are still unknown, they showed, however, great similarities in terms of molecular, solubility and functional group among the samples collected from the five cities. This indicates that it is probably acceptable to base results of toxicological testing on chlorinated organics from a limited number of drinking water sources.

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ABSTRACT

Since the previously unidentifiable non-volatile fraction of the chlorinated organics in drinking water is of significant health concern, the physical and chemical characteristics of the high molecular weight organics isolated from drinking water in five cities (namely: New Orleans, Philadelphia, Miami, Seattle, and Ottumwa) were compared. From the results of this study, they may be considered as part of a single group of natural organic compounds. Although the respective molecular structures of this group of compounds are still unknown, they showed, however, great similarities in terms of molecular, solubility and functional group among the samples collected from the five cities. This indicates that it is probably acceptable to base results of toxicological testing on chlorinated organics from a limited number of drinking water sources.

INTRODUCTION

Prior to 1975 most studies of the organic contaminants present in drinking water focused on those substances which were introduced by sources exogenous to the drinking water treatment process. When it was reported that trihalomethanes were produced during the chlorination of drinking water(1, 2), research began to focus more on the by-products of disinfection because this

step cannot be eliminated, as pathogenic bacteria must be controlled, and also because the identifiable products occurring most often in chlorinated drinking water were thetrihalomethanes. Thus, it was necessary to determine if these and other currently unidentifiable reaction by-products were of significant health importance. Of the greatest concern to this study would be the non-volatile fraction of the chlorinated organics which are known to contribute the bulk of the chlorinated compounds in drinking water and are previously unidentifiable while using gas chromatographic (GC) method for their analysis(3).

Because of the cost of isolating sufficient humic matter for all of the desire biological tests was prohibitive(4), samples of a commercially available humic acid were chlorinated and used in these tests. The work presented here was conducted to compare samples of this humic acid chlorinated under two different pH conditions with samples of humic-like material isolated from five chlorinated drinking waters in order to help decide if the results obtained from the biological testing of the former substance could be extrapolated to the later ones. The drinking water-derived material was obtained during an earlier study(5) from the drinking water of five cities in the United States, which at the time were thought to be representative of water

derived from sources containing various types and amounts of organic matter. Information regarding these water supplies is presented in Table 1. The organic matter isolated from some of these waters (by liquid-liquid extraction and XAD-2 adsorption from reverse osmosis concentrate) was found to be mutagenic(4). Most of the organic matter in these samples were polar acidic compounds and could not be chromatographed on silica gel even with methanol or in a gas chromatographic system(3). Because these samples were highly colored and acidic, they were thought to consist of humic or fulvic acid modified by the action of chlorine disinfectant. Efforts were thus made to isolate the humic and fulvic acid fractions and by-products using a series of solvent extractions to first remove the non-polar constituents with hexane and followed by ether extraction. The residual solids after ether extraction were then dissolved in acetone, and was found to contain mainly the polar acidic humic substances according to a previous study(6). This acetone soluble fraction of organic matter was subjected to a variety of physical and chemical tests to obtain as much information as possible on the similarities of the material isolated from the drinking water of the five cities which covered widely geographical sources. By so doing, it was judged to be possible to better estimate the similarities in biological activity that

may be expected with this material. If indeed they were similar in both physical/chemical characteristics and biological activities, then a large amount of high molecular weight drinking water organics can be isolated from one conveniently located water treatment plant for more extensive biological tests. This would greatly reduce the logistic problems of having to transfer qualified personnel and necessary equipment all over the country to collect representative samples for more in-depth studies on these materials.

In addition to characterizing the high molecular weight organic matter isolated from drinking water, samples of a naturally occurring aquatic humic acid and a commercially obtained humic acid chlorinated under both basic and neutral conditions in the laboratory were also characterized. This approach was taken as a best effort to determine if the presence of these chlorinated model humic acids in drinking water would constitute the same hazard to the health of the consumers as those isolated from actual drinking water samples(4). Therefore, it is important to be able to compare the similarity of the two types of material. Due to the complex nature of these substances, the tests reported here provide only the best means of comparing chlorinated organics obtained from widely different sources.

TABLE 1. Drinking Water Samples from Five Cities

City	Type of Raw Water Source	Suspected Types of Contaminants
Miami, FL	Ground	Uncontaminated*
New Orleans, LA	Surface	Industrial wastes
Ottumwa, IA	Surface	Agricultural runoff
Philadelphia, PA	Surface	Municipal wastes
Seattle, WA	Surface	Uncontaminated*

*Refers to no known contamination from municipal, agricultural, and industrial wastes -- but contamination presumably from decomposition products of natural origin.

MATERIALS AND METHODS

Concentration of Organic Matter from Drinking Water

Water samples were concentrated from drinking water of five cities (Table 1) by reverse osmosis using cellulose acetate membranes in conjunction with the Donnan softening process(7). The volume processed depended on the organic carbon content of the water and varied from 1,500 liters in Miami, Florida to 15,000 liters in Seattle, Washington. The permeate from this system was reprocessed by a nylon hollow fiber reverse osmosis loop(7). The two concentrates were sequentially extracted at neutral pH with pentane and methylene chloride and again at pH 2 with methylene chloride. After removal of residual solvent by purging with purified nitrogen the aqueous concentrates were passed through Amberlite XAD-2 columns.

The cross-sectional flow velocity through the resin columns was maintained at 10 cm/min. The columns were each rinsed with 2.5 bed volumes of 1 M HCl followed by 2.5 bed volumes of distilled water to remove metallic oxides and other inorganic constituents. Finally, the organic components were eluted with 2.5 bed volumes of 95% ethanol. Ethanol was selected as the eluent because it seemed to be the best choice as a solvent tolerated by animals used in proposed biological tests.

The ethanol was removed by vacuum distillation at 40°C and pressures of 203-208 mm mercury. The eluates from both columns were combined. In all cases, the majority of the organic matter was contained in the cellulose acetate membrane concentrate. These procedures have been more completely reported elsewhere(7).

Isolation of River Humic Substances

River water humic substances were isolated by this laboratory from the Satilla River using a modification of a previously described adsorption technique utilizing XAD-7 resin followed by elution with triethylamine(8). Excess triethylamine was removed in a rotary evaporator and the residual solution was continuously extracted with chloroform to remove neutral molecules. The resultant aqueous solution was reacted with BioRad AG 50WX8 cation exchange resin (H^+-form) and then freeze-dried. About 70% of Satilla River humic substances as measured by TOC was recovered. The elemental composition of the aquatic humus is as follows: 50.77% carbon, 3.81% hydrogen, 0.79% nitrogen and 2.28% ash. The characteristics of the aquatic humus based on oxidative degradation studies were reported by Reuter, et al.(9).

Commercially Available Humic Acids

A large quantity of the commercially available humic acids was purchased from Fluka AG (Buch, Switzerland) by the Health

Effects Research Laboratory (HERL) of EPA, Cincinnati, Ohio. This is a coal-base humic substance from West Germany.

Solvent Fractionation of Humic Substances

The organic matter from drinking water samples, Fluka humic acid and chlorinated Fluka humic, was fractionated using a series of solvents, e.g., hexane, ether and acetone (distilled-in-grass grade, Burdick and Jackson, Muskegon, MI) according to procedures developed by Kopfler(3). In general, these procedures involved the use of an ultrasonic bath to repeatedly extract the humic substances in 10-ml serum bottle with appropriate solvents. The insoluble fractions were separated in centrifuge tube and the supernatant layer was dewatered over anhydrous sodium sulfate (previously muffled at 550°C). The dehydrated solvent extracts were dried in an all-stainless steel-glass-Teflon evaporator using ultrapure nitrogen. The nitrogen gas flow rate was adjusted to 300 ml/min to minimize the loss of the volatile sample components. The evaporator was immersed in water bath set at 40°C.

Procedures for Chlorination of Humic Acids

Four grams of humic acid (Fluka AG, Lot #215315) were placed in a 500 ml beaker, to which 250-ml of distilled water were added. This was followed by the slow addition, with stirring, of

0.1 N NaOH until the pH was brought to 7.0. The solution was stirred for one hour after which the pH was checked and readjusted to 7.0 with 0.1 N NaOH if necessary. The sample was placed in an ultrasonic bath for two hours to aid dissolution and the pH was readjusted to 7.0 with 0.1 N NaOH if necessary. The sample beaker was covered and stirred overnight. The following morning the pH was checked and readjusted to 7.0 if necessary. The sample was then centrifuged in a Sorvall RC-2B centrifuge (DuPont, Wilmington, DE) at 16,300 G. The supernataut was decanted and diluted to 800 ml with distilled water. The solid residual was saved for gravimetric analysis.

The concentration of humic acid was determined by total organic carbon (TOC) analysis. TOC's for the humic stock solutions were normally 2.3 $\pm$ 0.1 g/l. Elemental analysis showed that the powdered humic substance was 52% carbon weight which means the theoretical maximum of TOC for an 800-ml solution prepared from 4.0g of humic substances is 2.6 g/l.

For chlorination of the humics at neutral pH, a solution of 11-13 g/l of chlorine at a pH of 7.5 was used. This solution was prepared by bubbling chlorine gas into a solution of 5.6 g NaOH in 500 ml of water. Bubbling was continued until the solution was within the pH range 7.5 $\pm$ 0.1. For chlorination of the humics

at high pH, a solution of 17-20 g/l of chlorine at a pH of 11.8 was used. This solution was prepared by bubbling chlorine gas into a solution of 11.3 g NaOH in 500 ml of water. Bubbling was continued until the solution was within the pH range 11.8 $\pm$ 0.2. The concentration of chlorine was determined by the starch-iodine method.

The chlorination of humics is carried out immediately after preparation of the chlorine water. Enough of the concentrated humic solution was added to a 250 ml amber bottle so that when diluted to 200 ml the TOC concentration would be 1.0 g/l. A ratio of one equivalent of chlorine (as Cl) per mole of carbon atoms (Cl:C=1:1) was most commonly used because at this ratio more than 99% of the chlorine equivalents are consumed in a 90 hour period. The solutions were added to the bottle in the order: humus, water, mix well, chlorine water, and a final mixing by shaking. The bottles were capped with Teflon backed septa.

Initially, at room temperature the chlorine reacted rapidly with the humus which was evidenced by the change in color or the solution from a thick black to transparent brown immediately after addition of the chlorine. A drastic drop of pH was observed after 17 hours (e.g., a drop from 7.0 to 2.6 while being chlorinated at neutral pH). Any storage after 90 hours was at 4°C for no more than 48 additional hours. The chlorinated samples were lipholized to dryness and stored in a refrigerator. The

acetone soluble fraction of each sample was subjected to the following analyses.

Elemental Analysis

The acetone fractions were analyzed for C, H and N by an automatic analyzer. Sulfur and the halogens (i.e., Cl and Br) were analyzed by combustion and subsequent titration. The following standard compounds were used for quality assurance (QA) purposes:

Acetanilide	C,H,N
Sulfanilamide	S
p-Chlorobenzoic acid	Cl
p-Bromobenzoic	Br

Spectral Studies

UV spectra were recorded in methanolic solution (40-60 ppm) on a Beckman Model 26 spectrophotometer (Irvine, CA), using matched quartz cells of 1-cm path length. A holmium oxide spectrum was recorded prior to the analysis of the samples for QA purposes.

Infrared spectra were recorded using a Beckman Acculab 6 spectrophotometer (Fullerton, CA). Pellets were prepared by torque pressing a finely ground mixture composed of 1-2 mg of sample and 100-500 mg of spectroanalytical KBr. The pellets were

translucent brown and showed good dispersion of the sample. Considerable attention was given to prevent moisture interference. The spectrum of a polystyrene film was recorded prior to the sample for QA purposes.

Fluorescence spectra were recorded in methanolic solution (40-50 ppm) on a Perkin-Elmer Model/204A spectrofluorometer (Norwalk, CT). Mutually maximized excitation and emission wavelengths were recorded in nm units. Water was used for QA purposes.

X-Ray Scattering

Each sample was analyzed by a low angle x-ray scattering technique in the laboratory of Wershaw and Pinkney(10).

Ultrafiltration (UF) and Size Exclusion Chromatograph (SEC)

The apparent molecular weight distribution of each sample was determined by sequential use of UF and SEC using a modification of a procedure proposed by Chian and DeWalle(11). Approximately 10 mg of a sample was dissolved in 200 ml of an alkaline buffer solution [pH = 10.4; 12.1 g of tris(hydroxymethyl)aminomethane per liter of"organic-free" water] and filtered through an UM05 UF membrane (Amicon, Burlington, MA) having a molecular weight cut-off at 500 Dalton. Membrane ultrafiltrations (UF) were performed in a static test

cell at a nitrogen pressure of 2.465 kg/cm^2. Fifty ml of retentate of each sample was collected. The UF retentate was further fractionated by SEC on Sephadex G-75 (100 x 1-cm I.D. glass column, bed height of 41 cm) (Pharmacia Fine Chemicals, Piscataway, NJ). The flow rate was maintained at 18 ml/h by means of a peristaltic pump (Milton Roy, River Beach, FL) and the column effluent was monitored by a variable UV detector (Perkin-Elmer, Norwalk, CT). The exclusion volume of the gel permeation column was found by chromatographing a mixture of blue-dextran (MW 2,000,000 Dalton) and phenol, and monitoring the column effluent with a variable UV detector (Model LC-65T, Perkin-Elmer, Norwalk, CT) at 230 mn.

Thermal Gravimetric Analysis (TGA) and Differential Thermal)Analysis (DTA)

Approximately 10 mg of accurately weighed sample were subjected to TGA and DTA. The percentage weight loss under a temperature program of 25°C/min was automatically recorded on a Mettler Thermoanalyzer 2 (Highstown, NY) and on a Perkin-Elmer TGS-2 Thermogravimetric System (Norwalk, CT). The mean weight loss of a $CaCO_3$ standard (3 runs) was 42.9% (theoretical 44%) with a precision of $\pm$ 1.0%. The minimum weight of 10 mg required for the analysis was determined by a test with aquatic humus on TGA.

Analysis of Acidic Functional Groups

a. Carboxyl Group Analysis

Carboxyl groups of the samples were determined by modifications of methods developed by Perdue, et al.(12). Approximately 10 mg of accurately weighted samples were used for this determination. Eleven analyses of an aquatic humus sample gave a mean value of 4.76 meq/g with a standard deviation (s) of 0.14 and a coefficient of variation (cv) of 2.9%.

b. Total Acidity (TA)

The procedure proposed by Schnitzer and Gupta (13) and Schnitzer and Kahn (14) was adopted with some modification for the determination of TA. All operations were carried out under N_2 atmosphere. Approximately 10 mg of accurately weighed samples were used for this determination. A blank experiment and six repetitive analyses from the Satilla River were performed. The mean value was 11.1 meq/g with s = 0.35 and cv = 3.2%.

Table 2 gives a list of samples investigated in this study.

RESULTS AND DISCUSSION

The results of the fractionation of the samples according to their solubility in hexane, ether and acetone are presented in Table 3. The recovery of acetone fractions of the five-city samples (samples 1-10) vary between 22.5 and 59.6% of the total

recovered weight; the ether fractions range between 20.3 and 56.2%; and the hexane fractions range between 2.5 and 4.8%. The two neutral-pH chlorinated Fluka-humic acid (CFH) samples gave a consistently lower percentage for three solvent fractions. As a result of this, the insoluble fraction of chlorinated Fluka humic acid becomes much higher (up to 83% as compared to a range of 1-17% with the drinking water sample). While the original Fluka humic acid gives the similar weight distribution in each of the solvent fractions as that of neutral-pH chlorinated Fluka-humic acid (CFH-1 and -2), the high-pH chlorinated Fluka-humic acid (CFH-3) shows a much greater difference in solubility with extraction solvents. This indicates that chlorination at neutral pH does not seem to alter significantly the nature of the model Fluka-humic acid. However, high-pH chlorination of Fluka-humic acid rendered all organically soluble fractions insoluble. More than 99% of the resulting organics upon high-pH chlorination of Fluka-humic acid are insoluble which indicate a severe alteration of the original Fluka-humic acid. Since our characterization studies use the acetone soluble fraction, further characterization of the high-pH chlorinated CFH-3 becomes impossible for lack of samples. Characterization of the acetone soluble fractions of other samples is described below.

TABLE 2. List of Samples Investigated in This Study

No.	Name	Detailed Descriptions
1	Miami 1A	See Table 1
2	Miami 1B	See Table 1
3	Miami 2	See Table 1
4	New Orleans 1A	See Table 1
5	New Orleans 2	See Table 1
6	Ottumwa 2	See Table 1
7	Ottumwa 1	See Table 1
8	Philadelphia 2	See Table 1
9	Philadelphia 1	See Table 1
10	Seattle	See Table 1
11	CFH-1	Low pH chlorination of Fluka H.A.
12	CFH-2	Low pH chlorination of Fluka H.A.
13	CFH-3	High pH chlorination of Fluka H.A.
14	Fluka Humic Acid	Fluka Lot #215315 coal base humus
15	Satilla River Humus (Aquatic Humus)	Naturally occurring surface water humus (see reference #9)
16	Chlorinated Aquatic Humus (Satilla River)	

Table 3. Fractionation of samples under investigation

	Sample	Initial Weight Fractionated	Fraction Soluble in: Hexane	Ether	Acetone	Insoluble Fraction	Percentage Recovered from Total Amount Fractionated
1	Miami 1A	2.0595	.0740 (4.4)	.5943 (35.1)	1.0115 (59.6)	.0148 (0.9)	82.2
2	Miami 1B	1.0424	.0447 (4.8)	.3637 (39.4)	.5111 (55.3)	.0044 (0.5)	88.6
3	Miami 2	4.8950	.0839 (3.8)	1.1041 (47.8)	1.0903 (47.2)	.0279 (1.2)	47.2
4	New Orleans 1A	1.8828	.0324 (2.5)	.6434 (50.0)	.5221 (40.5)	.0897 (7.0)	68.4
5	New Orleans 2	2.6387	.0418 (2.8)	.6711 (44.3)	.6298 (41.5)	.1734 (11.4)	57.5
6	Ottumwa 2	2.3787	.0523 (4.8)	.4138 (38.2)	.4425 (40.9)	.1734 (16.1)	45.5
7	Ottumwa 1	2.0784	.0305 (2.4)	.2544 (20.3)	.8403 (67.7)	.1202 (9.6)	60.3
8	Philadelphia 2	1.5244	.0280 (2.7)	.4808 (47.4)	.4575 (45.1)	.0469 (4.6)	66.4
9	Philadelphia 1	4.5167	.0509 (2.5)	.7937 (39.0)	1.1308 (55.5)	.0595 (2.9)	45.1
10	Seattle	.5007	.0065 (4.3)	.0859 (56.2)	.0344 (22.5)	.0259 (17.0)	30.5
11	CFH-1	1.0268	.0042 (0.4)	.0387 (3.9)	.1212 (12.3)	.8244 (83.4)	96.3
12	CFH-2	1.0524	.0043 (0.4)	.0415 (4.3)	.1225 (12.8)	.7922 (82.5)	91.3
13	CFH-3	1.03326	.000015 (0.01)	.00188(0.18)	.00417(0.41)	1.0047 (99.39)	97.8
14	Fluka Humic	1.01048	.0001 (0.01)	.0058 (0.63)	.13713(14.90)	.77733(84.46)	91.1

Weight in grams
*( ) Percentage of each fraction with respect to total amount recovered.

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Elemental Analysis

The elemental analyses are presented in Table 4. The atomic ratios H/C for all drinking water samples (no. 1-10) are between 1.28 and 1.39. This is comparable to humic acid derived from lake sediments. However, H/C ratios are much lower when compared to the chlorinated model humic substances (e.g., 1.04-1.08 for CFH-1 and CFH-2). Bromine is present in almost negligible quantities, whereas Cl varies between 0.3 and 2.4% and S between 0.9 and 2.7% in the drinking water organic matter. All fractions from drinking water show similar elemental composition. However, they differ from the elemental composition of the CFH samples in all respects especially chlorine content.

Spectral Studies

a. Ultraviolet Absorption

The acetone fractions derived from drinking water exhibit intense UV absorption between 208 and 214 nm, which may be attributed to unsaturated acids or esters. The CFH samples presented maximum absorption at 254-256 nm, whereas an aquatic humus sample derived from the Satilla River showed a maximum absorption at 234 nm. Therefore, apparent differences in UV spectra among all sample groups were found.

Table 4. Elemental analysis of acetone soluble fractions (%).

No.	Sample name	C	H	N	S	Cl	Br	Ash	H/C Atomic Ratio
1	Miami 1A	54.41	6.04	1.74	2.74	2.19	0.39	0.54	1.33
2	Miami 1B	57.51	6.11	2.27	2.07	1.45	0.24	0.91	1.28
3	Miami 2	57.20	6.19	1.85	1.13	1.42	0.36	1.06	1.30
4	N. Orleans 1A	56.11	6.50	1.47	0.87	2.24	0.14	1.91	1.39
5	N. Orleans 2	55.58	6.41	1.74	0.92	1.35	0.23	0.92	1.38
6	Ottumwa 2	58.31	6.73	2.95	0.94	0.30	<.10	0.46	1.38
7	Ottumwa 1	55.77	6.13	2.56	0.93	0.57	0.11	0.34	1.32
8	Philadelphia 2	53.67	6.13	1.99	1.83	1.56	0.20	0.45	1.37
9	Philadelphia 1	55.80	6.19	1.77	1.50	1.26	0.22	0.43	1.33
10	Seattle	52.83	6.03	2.11	1.04	2.02	<.10	0.97	1.37
11	CFH1	41.27	3.70	0.53	2.43	14.61	<0.5	2.51	1.08
12	CFH2	39.34	3.41	0.53	2.40	16.08	<0.5	2.80	1.04

b. Fluorescence Spectra

Fluorescence data are presented in Table 5. All samples derived from drinking water gave an emission maximum between 417 and 430 nm, while the excitation maximum ranged between 346 and 365 nm. Miami 1B showed an excitation maximum similar to the CFH samples, but quite different from the one exhibited by the aquatic humic substances from the Satilla River. The emission maximum of these samples (Table 5), however, was very similar.

c. Infrared Spectra

The IR spectra of all ten samples derived from drinking water are almost identical. Figure 1 shows a representative spectrum. For comparison the IR spectra of Satilla River aquatic humus and CFH are added. All samples exhibit broad bands in the 3400 cm^{-1} (-OH), 1720 cm^{-1} (C=O), and 1640 cm^{-1} (C=C)regions. However, the spectra from the ten drinking water samples and CFH samples show distinct differences from that of the Satilla River aquatic humus which absorbs sharply near 1600 cm^{-1}. This may suggest that the samples derived from drinking water are strongly altered by chlorination and/or oxidation. Further confirmation of the spectral dissimilarities should be pursued by analysis of chlorinated alteration products of humus.

Table 5. Fluorescence spectra of acetone soluble fractions

No.	Sample Name	Solvent	λ_{max} nm	
			Ex	Em
0	Aquatic Humus	MeOH	335	450
1	Miami 1A	MeOH	355	425
2	Miami 1B	MeOH	370	463
3	Miami 2	MeOH	352	426
4	N. Orleans 1A	MeOH	355	425
5	N. Orleans 2	MeOH	348	426
6	Ottumwa 2	MeOH	348	417
7	Ottumwa 1	MeOH	355	420
8	Philadelphia 2	MeOH	350	430
9	Philadelphia 1	MeOH	346	426
10	Seattle	MeOH	350	430
11	CFH1	MeOH	370	450
12	CFH2	MeOH	370	460

Figure 1. Infrared Spectra of: 1. Aquatic River Humus; 2. Chlorinated Aquatic River Humus; 3. Organics from Drinking Water (Philadelphia 1, #9) and 4. Chlorinated Fluka H.A. (CFH).

X-Ray Scattering

The results of the low-angle x-ray scattering analyses are reported in Table 6. By comparing the results (see Table 6) obtained in this study with those obtained by Thurman, et al.(15), based on the use of standards of polyglycols and polyacrylic acids, our aquatic samples along with the two neutral-pH chlorinated Fluka-humic acid all fall within the range reported by these authors (i.e., Rg ranges from 5.6 to 10.9 Angstrons which corresponds to molecular weights ranging from 800 to 3000 Dalton). A brief discussion on the molecular weights of humics as determined by the small-angle x-ray scattering method to those obtained by membrane ultrafiltration and Sephadex gel permeation methods will be presented in the following paragraph.

Ultrafiltration and Size Exclusion Chromatograph

The UF separation (i.e., drinking water organic matter, CFH) gave similar values for all samples. Eighty-nine percent or more of the initial weight was recovered in the retentate fraction (see Table 7). The subsequent analysis of the UF retentate by Sephadex G-75 showed differences in molecular weight distribution of the two sample categories. The typical elution patterns are shown in Figure 2, along with the calibration standard. The apparent molecular weights of drinking water derived samples

Table 6. X-ray scattering of acetone soluble fraction

No.	Name	R_g*	pH
1	Miami 1A	5.6	11
2	Miami 1B	7.6	9
3	Miami 2	7.4	11
4	New Orleans 1A	6.4	11
5	New Orleans 2	8.4	11
6	Ottumwa 2	5.4	10
7	Ottumwa 1	5.3	11
8	Philadelphia 2	7.2	11
9	Philadelphia 1	10.9	11
10	Seattle	Not done. Insufficient quantity.	
11	CFH 1	8.7	11
12	CFH 2	6.8	11

*Rg - Radius of gyration expressed in Angstrom units

Table 7. Ultrafiltration of acetone soluble fractions

No.	Sample	Original Wt (mg)	Retentate		Permeate		Total Recovery
			Wt (mg)	% Recovery	Wt (mg)	% Recovery	
1	Miami 1A	10.1	9.6	95	0	0	95
2	Miami 1B	10.3	12.1	117	0.3	3.6	120
3	Miami 2	10.1	9.9	98	1.8	18.1	116
4	New Orleans 1A	9.2	9.2	89	0	0	89
5	New Orleans 2	9.2	10.9	119	2.0	22.0	140
6	Ottumwa 2	9.9	9.1	92	1.0	9.7	102
7	Ottumwa 1	9.9	10.6	107	0	0	107
8	Philadelphia 2	9.7	10.4	107	1.6	16.0	124
9	Philadelphia 1	9.6	9.0	94	1.0	10.9	104
10	Seattle	Not done	Insufficient quantity				
11	CFH-1	11.0	11.1	101	0.6	5.5	107
12	CFH 2	11.0	11.1	101	0.6	5.5	107

Volume of original solution = 200 ml
Volume of retentate = 50 ml
Volume of permeate = 150 ml

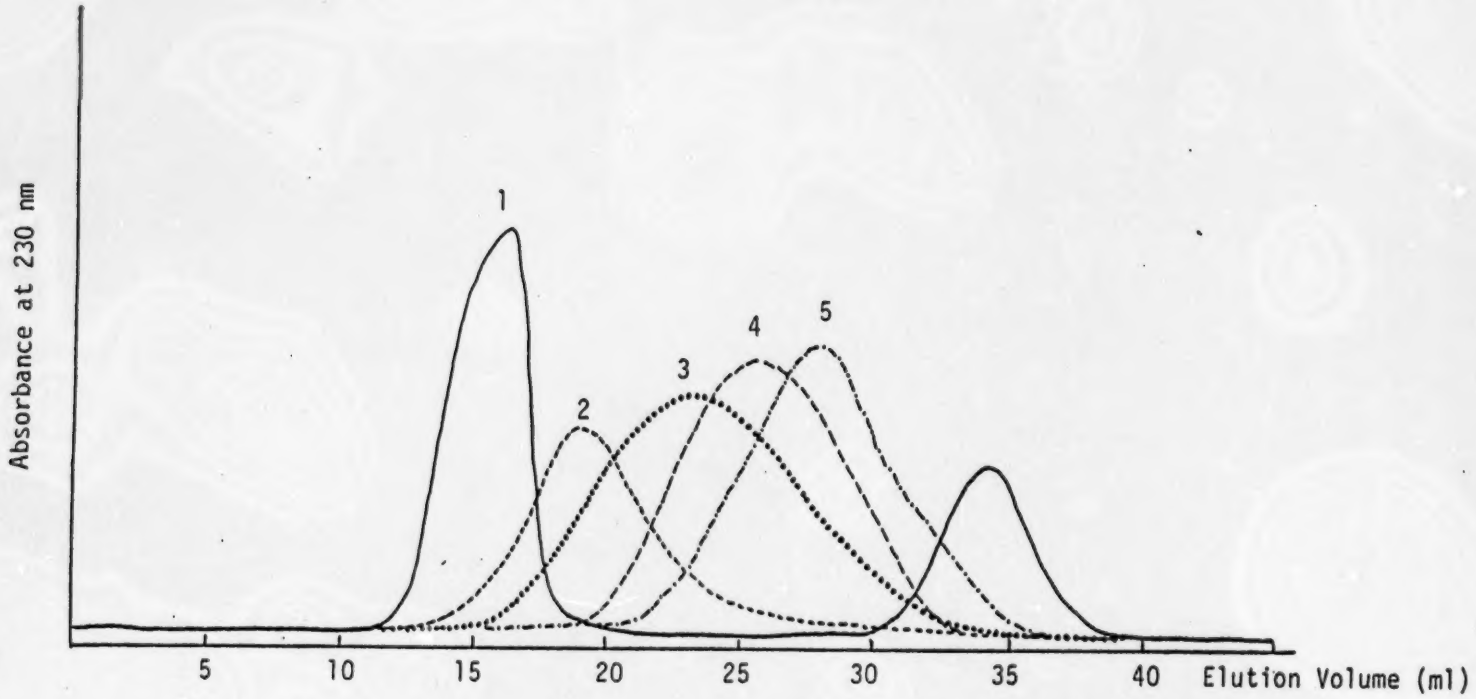

Figure 2. Gel Permeation Chromatography on Sephadex G75 of: 1. Blue Dextran and Phenol Standard; 2. Aquatic River Humus; 3. Chlorinated Fluka H.A. (CFH); 4. Chlorinated Aquatic River Humus; and 5. Organics from Drinking Water (Philadephia 1, #9).

range between 1000 and 5000 Dalton, whereas the CFH samples present a molecular weight of >5000 Dalton. According to the x-ray scattering results the sizes of CFH samples fall within those of the drinking water samples (Table 6). Therefore, no agreement between the SEC and the x-ray scattering results in terms of molecular sizes can be reached. However, Thurman, et al.(15) showed that Sephadex and ultrafiltration methods gave molecular weight of humic substances under 10,000 which are somewhat higher than those determined by the colligated properties methods (i.e., under 3000). These authors(15) also concluded that the data from low-angle x-ray scattering are similar to the measurements of colligated properties. This appears to be in good agreement with our study here (i.e., the ultrafiltration and Sephadex methods tend to give higher molecular weight determination than those obtained by the small-angle x-ray method).

Thermal Gravimetric Analysis (TGA) and Differential Thermal Gravimetric Analysis (DTA

TGA and DTA of the samples derived from drinking water gave similar thermal maxima temperatures (thermal maxima temperature were within 20°C) and thermal maxima curves. On the other hand, the CFH samples, although similar within themselves, presented widely different thermal maxima temperatures and curve shapes when compared with the ten samples derived from drinking water.

Representative DTA curves for these two sample categories are shown in Figure 3.

Acidic Group Analyses

[COOH] and total acidity values are reported in Table 8. Two distinct groupings can be recognized according to the two sample categories, although one CFH sample presents a [COOH] value (i.e., CFH-2) similar to at least three values found for the drinking water samples.

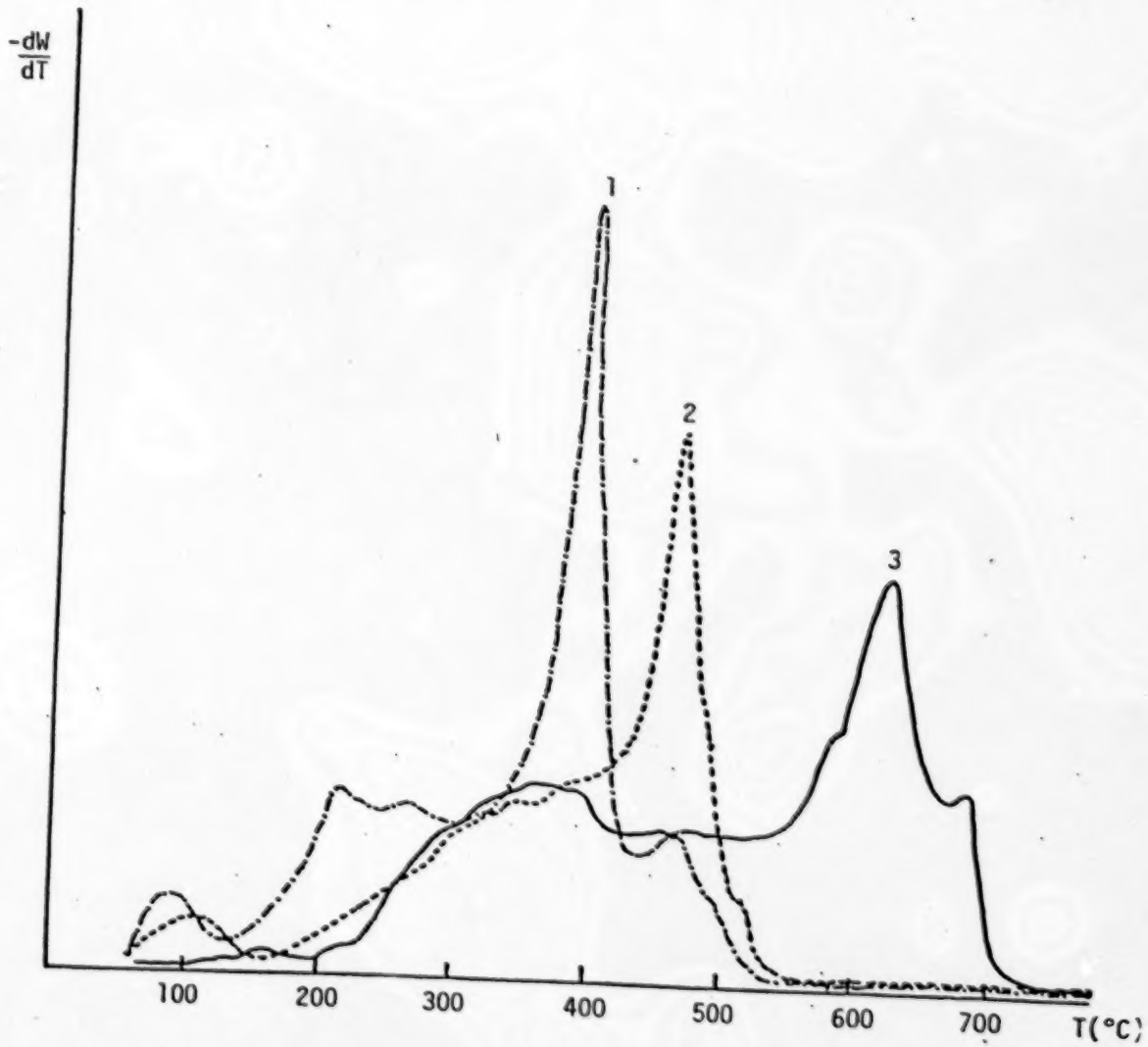

Figure 3. Differential Thermogravimetric Curves of: 1. Chlorinated Fluka H.A. , 2. Aquatic River Humus; and 3. Organics from Drinking Water (Ottumwa 1).

Table 8. Acidic functions of acetone soluble fraction

No.	Name	COOH meq/g	Total Acidity meq/g
1	Miami 1A	2.5	5.9
2	Miami 1B	2.4	6.1
3	Miami 2	2.7	5.8
4	New Orleans 1A	1.9	7.0
5	New Orleans 2	2.0	5.6
6	Ottumwa 2	2.3	5.3
7	Ottumwa 1	2.7	7.3
8	Philadelphia 2	2.3	5.2
9	Philadelphia 1	1.9	7.4
10	Seattle	Not done.	Insufficient quantity.
11	CFH 1	3.1	3.8
12	CFH 2	2.6	3.4

CONCLUSIONS

1. From the physical and chemical characteristics of all these samples, they may be considered as part of a single group of natural organic compounds. However, they are still unidentifiable in so far as their molecular structures are concerned.

2. The results of the samples from the five cities showed great similarities.

3. This indicates that it is probably acceptable to base results of toxicological testing on chlorinated humic from a limited number of drinking water sources.

4. Conversely, the coal-based Fluka humic acid may be too different from the aquatic humic matter in terms of molecular, solubility and functional group to serve as the best model for toxicological evaluation of drinking water-derived substance.

5. While comparing the physical and chemical characteristics of natural organics, care should be taken to ascertain that identical methods are being utilized in the isolation and concentration of these materials.

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